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(57) Abstract

Methods and compositions are provided for treating platelet-associated ischemic syndromes utilizing oligopeptides corresponding to regions of the platelet GPIIIa protein. The oligopeptides can be used to block platelet aggregation for numerous applications, and can serve in immunogen to raise receptor-specific antibodies.

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PLATELET BLOCKING PEPTIDES

Field of the Invention

The present invention relates generally to novel research, diagnostic and therapeutic agents and, more particularly, to compositions and methods useful, e.g., in the prevention and treatment of platelet-associated ischemic disorders. This application is a continuation-in-part-application of U.S.S.N. 213,641, filed June 30, 1988.

BACKGROUND OF THE INVENTION

Heart disease is the primary cause of death in most western societies. The most prevalent heart disease states are related to platelet-dependent ischemic syndromes, including, but not limited to, atherosclerosis and arteriosclerosis, acute myocardial infarction, chronic stable angina, unstable angina, transient ischemic attacks and strokes, peripheral vascular disease, arterial thrombosis, preeclampsia, embolism, restenosis following angioplasty, carotid endarterectomy, anastomosis of vascular grafts, and chronic cardiovascular devices (e.g., in-dwelling catheters). These syndromes represent a variety of stenotic and occlusive vascular disorders thought to be initiated by platelet activation on vessel walls.

Circulating platelets have been shown to play a central role in the blood vessel response to injuries, such as narrowing, plaque, foreign body presence (e.g., catheters) and the like. Very briefly, endothelial cell injury leads to a sequence of events including platelet adherence, platelet aggregation, and formation of microthrombi; and ultimate release of platelet granular components, including potent cellular mitogenic factors. These components assist in hemostasis, but can also induce undesirable events. Clinical manifestations of platelet related diseases can include any variety of atherosclerotic or s parately, thrombotic phenomenon.

Unfortunately, presently available therapeutic agents have not been proven to be of significant value in the broad treatment of platelet-associated ischemic disorders. Recently, attempts to inhibit thrombus formation have focused on blocking platelet adherence or aggregation. For example, short peptide sequences derived from both fibronectin and fibrinogen have been shown to inhibit platelet aggregation. These peptides are unlikely to be of therapeutic value, however, since adhesion of other cells (e.g., endothelial cells to the extracellular matrix) is also disrupted in their presence.

Other research has focused on blocking platelet aggregation at a crucial step in platelet recruitment i.e., fibrinogen binding. Mouse monoclonal antibodies against the fibrinogen receptor on platelets have been shown to reduce platelet aggregation in certain animal studies, but these are highly immunogenic, due to their size and foreign origin.

Thus, novel therapeutic treatment regimens for preventing or least mitigating undesirable thrombus formation are needed. In particular, therapeutic agents capable of blocking or inhibiting thrombus formation at specific locations would provide major therapeutic benefits.

Ideally, these agents will be potent, yet non-immunogenic to most patients. Also, the agent should be easy to administer, yet stable and economical to produce. Further, these agents should act transiently and be capable of functioning at the earliest stages of thrombus formation, without interfering with long-term hemostasis. The present invention fills these and other related needs.

SUMMARY OF THE INVENTION

Novel methods and compositions are provided for inhibiting platelet aggregation and other activities utilizing oligopeptides capable of specifically binding aggregation mediators, such as fibrinogen. The oligopeptides will typically comprise at least about five to twenty amino acids, and ar thus non-immunogenic and easy to produce, formulate and administer. These oligopeptides will be useful

in treating a variety of platelet-related diseases as well as in assays and for other uses.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention provides novel compositions and methods for treating platelet-associated ischemic syndromes by preventing or substantially inhibiting the formation of platelet aggregates. More specifically, oligopeptides mimicking regions of the receptor for platelet mediators, such as fibrinogen, are utilized to interfere with aggregation. In this regard, the term "blocking oligopeptide" indicates a peptide capable of binding to platelet mediators and interfering with activity of mediator receptors, particularly the receptors on stimulated platelets that bind fibrinogen, fibronectin and von Willebrand factor. These receptors are primarily responsible for recognition (i.e., binding) of the mediators and inducing their activities. The peptides can also act to impede the conversion of fibrinogen to fibrin for use in platelet or whole blood storage, and have a variety of other utilities, including as an immunogen to raise antibodies against the receptor.

The blocking oligopeptides can be used individually or together for the treatment regimens. Depending upon the particular use, the peptides may be labelled or unlabelled, conjugated to carriers, admixed with other compounds, or the like.

Typically, the peptides of interest will be deriv d from the amino-terminal portion of the platelet membrane glycoprotein (GP) IIIa, which is known to form a Ca^{2+}-dependent heterodimer complex with GPIIb (see, Phillips, et al., Blood, 71:831-843 (1988), which is incorporated herein by reference). This GPIIb/ IIIa complex constitutes, inter alia, the fibrinogen and fibrinonectin receptor on stimulated platelets. A biochemically and immunologically similar membrane glycoprot in complex has been shown to be present on endothelial cells as well.

Preferably, the peptides will comprise contiguous stretches within about the first 230 amino-terminal residues of GPIIIa. The first 200 residues are shown in Table 1 and residues 203-227 are listed in the text below (see, Fitzgerald, et al., J. Biol. Chem., 262:3936-3939 (1987), which is incorporated herein by reference). Table 2 presents five of the most preferred peptides of the present invention, each of which may include additional natively-associated amino acids (i.e., from the naturally-occurring GPIIIa sequence) or other additional components.

TABLE 1

1	G	P	N	I	C	T	T	R	G	V	10
11	S	S	C	Q	Q	C	L	A	V	S	20
21	P	M	C	A	W	C	S	D	E	A	30
31	L	P	L	G	S	P	R	C	D	L	40
41	K	E	N	L	L	K	D	N	C	A	50
51	P	E	S	I	E	F	P	V	S	E	60
61	A	R	V	L	E	D	R	P	L	S	60
71	D	K	G	S	G	D	S	S	Q	V	70
81	T	Q	V	S	P	Q	R	I	A	L	80
91	R	L	R	P	D	D	S	K	N	F	90
101	S	I	Q	V	R	Q	V	E	D	Y	110
111	P	V	D	I	Y	Y	L	M	D	L	120
121	S	Y	S	M	K	D	D	L	W	S	130
131	I	Q	N	L	G	T	K	L	A	T	140
141	Q	M	R	K	L	T	S	N	L	R	150
151	I	G	F	G	A	F	V	D	K	P	160
161	V	S	P	Y	M	Y	I	S	P	P	170
171	E	A	L	E	N	P	C	Y	D	M	180
181	K	T	T	C	L	P	M	F	G	Y	190
191	K	H	V	L	T	L	T	D	Q	V	200

TABLE 2

I	G S P R C D L K E N L L K D N C A P
II	A R V L E D R P L S D K G S G D S S Q V
III	D Q V T R F N E E V K K Q S V S R N R D
IV	E E V K K Q S V S R N R D A P E G G F D
V	V C D E K I G W R N D A S

Single Letter Code for Amino Acids

A-Alanine	G-Glycine	M-Methionine	S-Serine
C-Cysteine	H-Histidine	N-Asparagine	T-Threonine
D-Aspartic Acid	I-Isoleucine	P-Proline	V-Valine
E-Glutamate	K-Lysine	Q-Glutamine	W-Tryptophan
F-Phenylalanine	L-Leucine	R-Arginine	Y-Tyrosine

In a preferred mbodiment, the peptides will comprise at least a portion of peptide 203-227, so long as the peptide maintains the desired properties (e.g., fibrinogen binding, mimicking the fibrinogen binding site on GPIIIa, suitable for presenting antigenic determinant for raising antibodies against GPIIIa, and the like). For example, additional suitable peptides are derived from GPIIIa amino-terminal residues between about 203 and about 227, as follows:

203-227

N E E V K K Q S V S R N R D A P E G G F D A I M Q A

XXIII

N E E V K K Q S V S R N R D A P E G G

XXIV

S V S R N R D A P E G G F D A I M Q A

XXIII/XXIV (Overlap)

S V S R N R D A P E G G

The peptides of interest will include at least about 5 but generally less than about 50 amino acids, preferably 8 to 20, and usually fewer than about 35 amino acids. In each instance, the oligopeptide will ideally be as small as possible, while still maintaining substantially all of the desired activity, e.g., blocking activity. In some instances, it may be desirable to join two or more oligopeptides from different regions, which separately or together provide the desired activities. The peptides may, of course, be fused to other proteins or molecules with desired activities (e.g., thrombolytic activity).

It will be readily appreciated by skilled artisans that the peptides employed in the subject invention need not be identical to any particular of the most preferred polypeptide sequences shown in Table 2 or listed above in the text, so long as the subject compound

is able to provide blocking or other desired activities at a sufficient level. Therefore, the peptides may be subject to various changes, such as insertions, deletions, substitutions, eith r cons rvative or non-conservative, to provide for certain advantages in their use. Conservative substitutions are typically within groups, such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; Phe, Tyr; and Nor (norleucine), Met. Usually, the final sequence will not differ by more than about 10 to 40 percent from the naturally-occurring receptor sequence, except e.g., where additional amino acids may be added at either terminus for other utilities, including conjugation to carriers.

The peptide in which amino acid sequence has been modified by the substitution, addition or deletion of amino acid residues should retain substantially all of the blocking activity of the unmodified peptides, which may be conveniently measured by various assay techniques disclosed herein. Also, the small d-isomer form of one or more of the amino acids may be used, as desired, to modify biological properties, such as activity, rate of breakdown, etc.

Other modifications to the peptides can include the addition of one, two or more amino acids to the termini, such as to provide facilitated linking capability or to further modify the oligopeptide's physical or chemical properties. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, may be introduced at the C- or N-terminus of the oligopeptide. Cysteine is particularly preferred to facilitate covalent coupling to other peptides, to form polymers by oxidation or to form internal bridges within the oligopeptide.

Additionally, the oligopeptide sequences may differ from the natural sequences by modification according to a variety of well known biochemical reactions, such as amino-terminus acylation, e.g., acetylation, thioglycolic acid amidation, terminal-carboxy amidation (such as with

ammonia or methylamine) to provide stability, increased hydrophobicity or for polymerization.

The oligopeptides of the present invention can be prepared in a wide variety of ways. The peptides, because of their relatively short size, may be synthesized in solution or on a solid support in accordance with conventional techniques. See, for example, Stuart and Young, Solid Phase Peptide Synthesis, 2d Edition, Pierce Chemical Co. (1984); and Tam, et al., J. Am. Chem. Soc. 105:6442 (1983). Various automatic synthesizers are commercially available and can be used in accordance with known protocols. Also, specialty peptides can be ordered from a variety of commercial sources such as Bio Search, Inc., San Rafael, California, or Peninsula Laboratories.

Alternatively, hybrid DNA technology may be employed, where a synthetic gene is prepared utilizing single DNA strands coding for the desired oligopeptides, or substantially complementary strands thereof. Where the single strands overlap, they can be brought together in an annealing medium for hybridization. The hybridized strands may then be ligated to form the complete gene, and, by choice of appropriate termini, the gene may be inserted into expression vectors. See, for example, Maniatis, et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory (1982), which is incorporated herein by reference.

As desired, fragments from the naturally occurring sequence may be employed for expression of the peptide fragments, and conservative base changes can be incorporated, such that the modified codons code for the same amino acid. Similarly, non-conservative changes can be incorporated where the resulting amino acid sequence is to be changed as discussed previously.

The coding sequence may be extended at either of the 5'- or 3'-terminus, or both termini, to extend the peptide, while retaining its blocking sites. The extension may provid for an arm for linking, e.g., to a label, such as an enzyme, for joining two or more peptides together in

the same chain, for providing antigenic activity, convenient restriction sites for cloning, or the like.

The DNA sequences or fragments ther of are typically placed in expression vectors for ultimate transfection into a suitable host. See, Winnacker, E., From Genes to Clones, VCH Publishers, New York (1987), which is incorporated herein by reference. The host can be cultivated to enhance expression of the desired polypeptides, which then may be purified in accordance with standard techniques.

It is not known whether the subject polypeptides occur naturally. The present invention, thus, relates particularly to the non-naturally-occurring forms of receptor fragments, such as in isolated or purified, or substantially pure form. Typically, the peptides will be in a substantially different environment than in the naturally-occurring state, for example, in admixture with pharmaceutical carriers or the like. The synthetically or recombinantly produced peptides and their salts are preferred forms.

Suitable salts of the peptides according to the present invention are pharmaceutically acceptable non-toxic salts. The peptides can form acid addition salts, for example with inorganic acids, especially mineral acids. For peptides having at least one carboxy group and at least one basic group, for example an amino group, internal salts can be formed. Also, for peptides containing at least one free carboxy group, especially those having more carboxy groups than basic groups, metal ammonium salts, such as alkyline metal and alkyline earth metal salts, can be produced. Of course, for isolation and purification one may utilize pharmaceutically unsuitable salts, but only the pharmaceutically acceptable non-toxic salts should be employed for therapeutic use.

A "therap utically ffective dose" of the oligopeptides of the present invention will be an amount sufficient to diminish platelet aggregation below a l vel associated with pathological events, such as platelet

ischemic syndromes, and yet allow adequate hemostasis. If desired, the oligopeptides may be a co-administered with other agents, such as heparin, aspirin, dipyridamole, tissue plasminogen activator, streptokinase, urokinase, sulfinpyrazone, suloctidil, the peptide Arg-Gly-Asp-Ser, and/or antibodies reactive with the IIb/IIIa receptor. See, e.g., Harker, L., Am. J. Cardiol., 60:208-288 (1987), which is incorporated herein by reference.

By way of example and not limitation, the inhibition of platelet activities by interfering with the binding of fibrinogen to the IIb/IIIa receptor may find use in a wide variety of therapeutic settings, such as the following:

A. Prevention or abortion of the arterial thrombus formation.

In addition to treatment of unstable angina and arterial emboli or thrombosis, the oligopeptides are useful in the treatment or prevention of myocardial infarction (MI) and mural thrombus formation post MI. For brain-related disorders, treatment or prevention of transient ischemic attack and treatment of thrombotic stroke or stroke-in-evolution are included.

B. Prevention of platelet aggregation, embolization or consumption in extracorporeal circulations.

These uses include improving renal dialysis, cardiopulmonary bypasses, hemoperfusions, and plasmapheresis.

C. Prevention of platelet aggregation, embolization, or consumption associated with intravascular devices.

Improved utility of intraaortic balloon pumps, ventricular assist devices, and arterial catheters also results.

D. Treatment or prevention of venous thrombosis.

The oligopeptides will also be useful in deep venous

thrombosis; IVC, renal vein, or portal vein thrombosis; and pulmonary embolism.

E. Hematologic applications.

Various disorders involving platelet consumption, such as thrombotic thrombocytopenic purpura are treatable.

In addition, the peptides of the present invention may be used in numerous non-therapeutic applications where inhibiting platelet aggregation is desired. For example, improved platelet and whole blood storage can be obtained by adding sufficient quantities of the peptides, the amount of which will vary depending upon, inter alia, the length of proposed storage time, the conditions of storage, the ultimate use of the stored material, etc.

The peptide dosage can range broadly depending upon the desired affects and the therapeutic setting. Typically, dosages will be between about 0.01 and 10 milligrams per kilogram, preferably between about 0.01 to 0.1 milligrams per kilogram, body weight. Administration is preferably parenteral, such as intravenous on a daily basis for up to a week or as much as one to two months or more, all of which will vary with the peptide's size. If the peptides are sufficiently small (e.g., less than about 8-10 amino acid residues) other routes of administration can be utilized, such as intranasally, sublingually, or the like.

Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride or the like. In addition, if desired, the injectable pharmaceutical compositions may contain minor amounts of non-toxic auxiliary substances, such as wetting agents, pH buffering agents, and the like. If desired, absorption

enhancing preparations (e.g., liposomes) may be utilized.

Suitable in vitro assays for determining th peptides' blocking capability can be performed using standard methods (see, Gartner and Bennett, J. Biol. Chem., 260:11891-11894 (1985), which is incorporated herein by reference). Platelets are first isolated from healthy human donors, preferably by gel filtration to avoid subjecting cells to the rigors of centrifugation. Purified fibrinogen and calcium are added to the gel-filtered platelets, which are then placed in an aggregometer for platelet aggregation measurements (see, Ingerman-Wojenski and Silver, Thromb. Haemstas., 51:154-156 (1984) and Glazier, Am. Clin. Prod. Dev. (April 1987), both of which are incorporated herein by reference). Utilizing standard platelet aggregation stimuli, such as thrombin, ADP, collagen or epinephrine, the synthetic peptides are added, and aggregation inhibition measured. Thereafter, positive testing peptides are tested directly for the ability to inhibit fibrinogen binding to platelet receptor. In the subsequent assay, fibrinogen is purified from human plasma using standard technology, and labelled (e.g., with I^{125}). The binding of fibrinogen to the stimulated platelets is then determined (see, Bennett, et al., J. Clin. Invest., 64:1393-1401 (1979), which is incorporated herein by reference).

When the peptides of the present invention are polymerized to each other or conjugated to carriers, they are particularly useful for raising antibodies (polyclonal or monoclonal) against the GPIIIa portion of the receptor. Where different peptides are used in the antigenic mixture, it is possible to induce the production of antibodies immunoreactive with several epitopes of the glycoprotein.

The subject oligopeptides may be employed linked to a soluble macromolecule, typically not less than about 5kD, carrier. Conveniently, the carrier may be a poly (amino acid), either naturally-occurring or synthetic, to which antibodies are likely to be encountered in human serum. Examples of such carriers are poly-Lysine,

hemocyanin, thyroglobulin, albumins, such as bovine serum albumin, tetanus toxoid, etc. As desired, one or more different oligopeptides of the present invention may be linked to the same macromolecule.

The manner of linking the oligopeptide with the carrier is conventional, such reagents as p-maleimidobenzoic acid, p-methyldithiobenzoic acid, maleic acid anhydride, succinic acid anhydride, glutaraldehyde, etc. The linkage may occur at the N-terminus, C-terminus or at a site intermediate to the ends of the molecule. The peptide may be derivatized by linking, may be linked while bound to a solid support, or the like, to form antigens or for other uses.

Numerous methodologies are presently known in the art for producing monoclonal antibodies (MoAbs) to the peptides. See, e.g., Goding, Monoclonal Antibodies; Principles and Practice, Academic Press, 2d Ed. (1986), which is incorporated herein by reference. Less preferred forms of immunoglobulins may be produced by methods well known to those skilled in the art, e.g., chromatographic purification of polyclonal sera to produce substantially monospecific antibody populations.

A commonly employed process for producing MoAbs involves fusion, under appropriate conditions, of an immortalizing cell line with a B-lymphocyte which produces the desired antibody. Immortalizing cell lines are well known in the art, and include lines which are of mammalian origin, typically of murine, rat, bovine, or human origins. They are generally tumor lines or cells obtained by transforming a normal cell line with, for example, Epstein Barr virus. Any immortalizing line can be used to prepare the hybridoma of the invention.

Similarly, techniques for obtaining the appropriate lymphocytes from mammals injected with the target antigen are well understood. Generally, either peripheral blood lymphocytes and cells of human origin are desired, or spleen cells, if mammalian non-human sourc s are employed. A subject mammal is injected with repeated dosages of th

purified antigen, and the mammal is permitted to generate the d sired antibody producing spleen cells or blood lymphocytes before these are harvest d for fusion with the immortalizing line.

Techniques for fusion are also well known in the art and, in general, involve mixing the cells with a fusing agent such as, most commonly, polyethylene glycol. Preparation of a hybridoma by fusing these two types of cells is, by now, well known in the art. Successful hybridoma formation is assessed and selected by standard procedures, such as, for example, HAT selection. From among successful hybridomas, those secreting the desired antibody are selected by assaying the culture medium for their presence. Ordinarily, this is done using immunoreaction based assays, including, without limitation, Western Blot, Elisa, or RIA assays. The antibodies can be recovered from the medium using standard protein purification techniques.

Antibodies reactive with the oligopeptides of the present invention will find various diagnostic uses, e.g., in detecting the presence of the GPIIb/IIIa receptor on various cell populations in accordance with techniques well-known to those skilled in the art. Further, the antibodies can serve as thrombus imaging agents, when labelled with ^{131}I; ^{99}Tc; and the like.

Experimentally, the following assay was used to assist in identifying peptides within the GPIIIa protein that inhibit the binding of fibrinogen to GPIIb-IIIa. Purified GPIIb-IIIa was added to the bottoms of 96-well microtiter plates. Biotinylated fibrinogen was then added, in the presence or absence of possible inhibitors of fibrinogen binding to GPIIb-IIIa, and allowed to incubate for 3 hours at 30'C. The plates were then washed, and an anti-biotin antibody conjugated to alkaline-phosphatase added. After a 30 minute incubation, the plate was again washed, and a substrate for alkalin phosphatase (p-nitrophenyl phosphate) added. The amount of fibrinogen bound to GPIIb-IIIa was quantitated by reading the optical

density of each well at 405 nm.

Using this assay, the region extending from the asparagine at amino acid #203 to the alanine at amino acid #227 of the GPIIIa NH_2-terminus was identified as blocking. In particular, within this sequence a 12 amino acid peptide (XXIII/XXIV (overlap)) was found to inhibit fibrinogen binding to GPIIb-IIIa. Moreover, a polyclonal antibody raised against both peptide XXIII, encompassing an amino-terminal portion of peptide 203-227, inhibited fibrinogen-binding to GPIIb-IIIa in the plate assay and partially inhibited the ADP-induced aggregation of human platelets.

Without intending to be bound to a particular theory, it is believed that the above peptides are binding to fibrinogen, mimicking the binding site on GPIIIa. Evidence for this mechanism of action includes the finding that pre-incubation of fibrinogen with the peptides greatly potentiated the inhibition of fibrinogen binding to GPIIb-IIIa. Thus, after infusion, the subject peptides would bind to fibrinogen at the GPIIb-IIIa binding site, and render the coupled fibrinogen incapable of binding to activated platelets. This provides specific inhibition of platelet aggregation, which will have therapeutic benefits, as in settings such as unstable angina or immediately following angioplasty. The peptides are also extremely useful in raising antibodies capable of blocking the interaction between GPIIIa and fibrinogen.

The identification of the GPIIIa region from peptide 203-227 as a fibrinogen-binding domain provides a means of screening large numbers of compounds (e.g., antibodies, organics, and the like) for potential use as inhibitors of fibrinogen-binding to GPIIb-IIIa on platelets. This is accomplished by coating the bottoms of 96-well microtiter plates with one or more of peptide XXIII, peptide XXIV or the XXIII/XXIV (overlap) peptide, then mixing in a sample of labelled compounds (such as radiolabeled antibiotics or organic compounds) and determining which bind to the selected p ptide(s). Alternativ ly, an antibody specific for the compound can be

included to determine if the screened compound binds to the peptid (s). Compounds which are shown to bind to one or more of these peptides are then tested directly for their ability to inhibit fibrinogen binding to GPIIb-IIIa.

These peptides will also find use in determining which region of fibrinogen binds to GPIIb-IIIA. For example, the XXIII/XXIV (overlap) peptide is coupled to Affi-Gel 10 beads (BioRad, Richmond, Ca) and a proteolytic digest of fibrinogen passed over the column. Fragments of fibrinogen which bind to the immobilized peptide (and not control peptides) are sequenced from the N-terminal) to identify GPIIb-IIIa binding sites within fibrinogen. These peptides are then synthetically manufactured and examined for their ability to inhibit fibrinogen binding to GPIIb-IIIa as detailed above.

From the foregoing, it will thus be appreciated that in addition to the peptides' blocking characteristics, the peptides provide guidance on what regions of GPIIIa are important for binding to fibrinogen. Thus, structural analogs can be provided which remain blocking, but at significantly lower amounts (i.e., concentration levels). These analogs can also be designed specifically for economic production, storage and administration.

Although the present invention has been described in some detail by way of illustration for purposes of clarity of understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the amended claims.

WE CLAIM:

1. A composition comprising at least about five continuous amino acids from an oligopeptide of the formula:

I.

Gly-Ser-Pro-Arg-Cys-Asp-Leu-Lys-Glu-Asn-Leu-Leu-Lys-Asp-Asn-Cys-Ala-Pro-Z;

II.

Ala-Arg-Val-Leu-Glu-Asp-Arg-Pro-Leu-Ser-Asp-Lys-Gly-Ser-Gly-Asp-Ser-Ser-Gln-Val-Z;

III.

Asp-Gln-Val-Thr-Arg-Phy-Asn-Glu-Glu-Val-Lys-Lys-Gln-Ser-Val-Ser-Arg-Asn-Arg-Asp-Z;

IV.

Glu-Glu-Val-Lys-Lys-Gln-Ser-Val-Ser-Arg-Asn-Arg-Asp-Ala-Pro-Glu-Gly-Gly-Phe-Asp-Z; and

V.

Val-Cys-Asp-Glu-Lys-Iso-Gly-Try-Arg-Asn-Asp-Ala-Ser-Z;

203-227.

Asn-Glu-Glu-Val-Lys-Lys-Gln-Ser-Val-Ser-Arg-Asn-Arg-Asp-Ala-Pro-Glu-Gly-Gly-Phe-Asp-Ala-Ile-Met-Gln-Ala-Z;

XXIII.

Asn-Glu-Glu-Val-Lys-Lys-Gln-Ser-Val-Ser-Arg-Asn-Arg-Asp-Ala Pro-Glu-Gly-Gly-Z;

XXIV.

Ser-Val-Ser-Arg-Asn-Arg-Asp-Ala-Pro-Glu-Gly-Gly-Phe-Asp Ala-Ile-Met-Gln-Ala-Z; or

XXIII/XXIV.

Ser-Val-Ser-Arg-Asn-Arg-Asp-Ala-Pro-Glu-Gly-Gly-Z;

wherein Z, if present, is OH and the oligopeptide has less than about 50 natively associated amino acids.

2. A composition according to Claim 1, wherein the oligopeptide is a salt.

3. A composition according to Claim 1, wherein the carboxy-terminal amino acid is free or amidated.

4. A composition according to Claim 1, wherein the amino-terminal amino acid is free or acetylated.

5. A composition according to Claim 1, wherein the oligopeptide is conjugated to a carrier.

6. A composition according to Claim 5, wherein the carrier is a protein.

7. A pharmaceutical composition comprising an oligopeptide of Claims 1, 2, 3, or 4.

8. A pharmaceutical formulation comprising a therapeutically effective dose of an oligopeptide according to Claims 1, 2, 3, or 4 in combination with a pharmaceutically acceptable carrier.

9. An oligopeptide comprising from about five to fifty contiguous amino acids of the 230 N-terminal residues of GPIIIa in combination with a pharmaceutically acceptable carrier.

10. A method of treating a patient suspected of having a platelet-associated ischemic syndrome comprising administering to the patient a therapeutically effective dose of an oligopeptide according to Claim 1 or 9 or a pharmaceutically acceptabl salt ther of.

11. A method of inhibiting platelet aggregation with a blocking peptide capable of inhibiting the binding of fibrinogen to a GPIIb/IIb receptor on the platelet, wherein the blocking peptide comprises at least about five contiguous amino acids from peptides I-V, 203-227, XXIII, XXIV, and XXIII/XXIV (overlap).

12. A method for improving the storage of a preparation of whole blood or platelets comprising admixing a blocking peptide into the preparation.

13. A composition comprising antibodies reactive with an epitope defined by at least about five contiguous amino acids of peptides I-V, 203-227, XXIII, XXIV and XXIII/XXIV (overlap).

14. A composition according to Claim 13, wherein the antibodies are monoclonal.

15. A method of screening compounds capable of inhibiting fibrinogen binding to GPIIb/IIIa, said method comprising mixing a sample of the compounds with at least one peptide selected from the group of peptides 203-227, XXIII, XXIV and XXIII/XXIV (overlap), and determining the level of binding between the sample and the peptide.

16. A methods according to Claim 14, wherein the peptide is coated on a microtiter plate.

17. A method according to Claim 14, wherein the sample comprises radiolabelled antibiotics.

18. A methods according to Claim 14, wherein the sample is mixed with antibodies and one or more peptides.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/02893

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. Cl.4-C07K 7/08,10; A61K 37/02
U.S. Cl.-530/326,324; 514/13,12

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/326,324; 514/13,12

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	The Journal of Biological Chemistry, vol. 262, No. 9, issued 25 March 1987, FITZGERALD, "Protein Sequence of endothelial glycoprotein IIIa derived from a cDNA clone", pp. 3936-3939, See the entire document.	1-11
A	Biochemistry, vol. 11, No. 24, issued 1972, PHILLIPS, "Effect of trypsin on the exposed polypeptides and glycoproteins in the human platelet membrane", pp. 4582-4588, See the entire article.	1-11

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 October 1989	30 OCT 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/USA	Teresa D. Wessendorf

Form PCT/ISA/210 (second sheet) (Rev.11-87)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	Th Journal of Biological Chemistry, vol. 260, No. 3, issued 10 February 1985, PARISE, "Platelet membrane glycoprotein IIb-IIIa complex incorporated into phospholipid vesicles", pp. 1750 -1756. See the entire article.	1-11

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Proc. Natl. Acad. Sci. USA, vol. 83, issued August 1986, PLOW, "Immunologic relationship between platelet membrane glycoprotein GPIIb/IIIa and cell surface molecules expressed by a variety of cells", pp. 6002-6006, See the entire document.	1-11
A	Proc. Natl. Acad. Sci. USA, vol. 83, issued November 1986, CHARO, "Platelet glycoproteins IIb and IIIa: evidence for a family of immunologically and structurally related glycoproteins in mammalian cells", pp. 8351-8355, See entire document.	1-11
A	Cell, vol. 44, issued 28 February 1986, RUOSLAHTI, "Arg-Gly-Asp: a versatile cell recognition signal", pp. 517 and 518, See the entire document.	1-11
A	Cell, vol. 48, issued 27 February 1987, KISHIMOTO, "Cloning of the beta subunit of the leukocyte adhesion proteins: homology to an extracellular matrix receptor defines a novel supergene family", pp. 681-690, See the entire document.	1-11
P,A	US, A, 4,789,734, 6 December 1988, (PIERSCHBACHER), See the entire document.	1-11